Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/GB05/000151

International filing date: 17 January 2005 (17.01.2005)

Document type: Certified copy of priority document

Document details: Country/Office: GB
Number: 0401008.8
Filing date: 17 January 2004 (17.01.2004)

Date of receipt at the International Bureau: 02 March 2005 (02.03.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

PCT/GB 2005/000151

INVESTOR IN PEOPLE

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

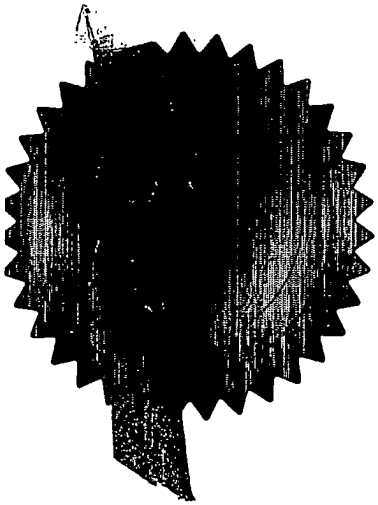
I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

I also certify that the application is now proceeding in the name as identified herein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.



Signed Andrew Gersey

Dated 14 February 2005

INVESTOR IN PEOPLE

GB 0401008.8

By virtue of a direction given under Section 30 of the Patents Act 1977, the application is proceeding in the name of:

THE UNIVERSITY OF MANCHESTER,
Oxford Road,
MANCHESTER,
M13 9PL,
United Kingdom

[ADP No. 07792138001]

Patents Form 1/77

Patents Act 1977
(Rule 16)

The Patent Office

1/77

19JAN04 E866154-1 C50092
P01/7700 0.00-0401008.8 NONE

17 JAN 2004

THE PATENT OFFICE
L
17 JAN 2004
NEWPORT

Request for grant of a patent

(see the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
Gwent NP10 8QQ

1. Your reference — AH/PN/P89548PGB

2. Patent application number *(The Patent Office will fill in this part)* — 0401008.8

3. Full name, address and postcode of the or of each applicant *(underline all surnames*

THE UNIVERSITY OF MANCHESTER INSTITUTE OF
SCIENCE AND TECHNOLOGY
PO BOX 88, SACKVILLE STREET
MANCHESTER M60 1QD

SECTION 20 (1977 ACT) APPLICATION FILED 4/11/04

Patents ADP number *(if you know it)* 00773762001

If the applicant is a corporate body, give the country/state of its incorporation — UNITED KINGDOM

4. Title of the invention — Drug Delivery System

5. Name of your agent *(if you have one)* — Marks & Clerk

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

43 Park Place
Leeds
LS1 2RY

Patents ADP number *(if you know it)* — 18013✓

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day/month/year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day/month/year)*

8. Is a statement of Inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d)) — YES

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	-
Description	22
Claim(s)	4 DL
Abstract	-
Drawing(s)	3+3

10. If you are also filing any of the following, state how many against each item.

Priority documents	-
Translations of priority documents	-
Statement of Inventorship and right to grant of a patent *(Patents Form 7/77)*	-
Request for preliminary examination and search *(Patents Form 9/77)*	-
Request for substantive examination *(Patents Form 10/77)*	-
Any other documents *(Please specify)*	-

11. I/We request the grant of a patent on the basis of this application.

Signature Marks + Clerk Date 17/01/04

MARKS & CLERK

12. Name and daytime telephone number of person to contact in the United Kingdom DR A HUTTER - 0113 3895602

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) *If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.*
b) *Write your answers in capital letters using black ink or you may type them.*
c) *If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.*
d) *If you have answered 'Yes' Patents Form 7/77 will need to be filed.*
e) *Once you have filled in the form you must remember to sign and date it.*
f) *For details of the fee and ways to pay please contact the Patent Office.*

DRUG DELIVERY SYSTEM

The present invention relates to drug delivery systems and particularly, although not exclusively, to drug delivery systems which exploit the PepT1 pathway. More specifically, the invention relates to peptide drug carrier molecules, and peptide drug carrier-drug conjugates which are transported across the wall of the gut into the blood by PepT1 protein, and uses thereof in medicine. The invention further extends to methods of synthesising such peptide drug carriers.

A large proportion of drugs used in medicine, that are orally administered, are subject to structural modification and, in some cases, substantial degradation in the gut, and this can often lead to a decrease in the biological activity of the drug. Accordingly, the medicinal efficacy of such drugs can be limited when taken orally. Furthermore, a large number of drugs that exhibit medicinal properties cannot be administered to a patient orally because they have poor solubility, or they are unable to diffuse across the wall of the gut into the bloodstream. Therefore, unfortunately, such drugs are either totally rejected for use in medical treatment, or have to be administered to patients by intravenous injection, which is invasive and has associated problems with many patients. Accordingly, there is a need to develop mechanisms by which drugs, which are either injected intravenously, or are not used at all, can be administered orally and transferred into the blood via the gut without any loss in biological activity. In addition, there is a need to improve the transportation of drug molecules across the wall of the gut in respect of those drugs which are currently administered orally, but which show decreased or low levels of medicinal activity.

PepT1 is a trans-membrane protein that is highly expressed in the jejenum region of the small intestine, and transports small peptides, such as the breakdown products of protein in food, across the wall of the gut into the bloodstream. PepT1 transports di-peptides and tri-peptides across the gut wall efficiently. Substrate transportation by PepT1 is driven by proton and electrochemical gradients and provides a mechanism by which peptidic drugs such as β-lactam antibiotics, and ACE inhibitors, for example, Captopril, can be orally absorbed by patients. Accordingly, drugs that do not naturally diffuse across the villi of the small intestine, or those

which have poor solubility, and which are only administerable by intravenous injection may be made orally administrable by transporting them across the wall of the gut into the bloodstream via the PepT1 pathway. In addition, the PepT1 pathway may also be exploited to improve the transportation of drugs which are currently administered orally, but which show decreased levels of biological activity, for example, because they are modified or degraded in the gut before they are transported into the blood.

Therefore, it is an aim of embodiments of the present invention to address the above problems and to provide a drug delivery system, which could be made available to the medical community, so that drugs, which are normally administered orally but which exhibit reduced or low levels of medicinal activity can have their performance improved. In addition, the drug delivery system could also enable drugs that are administered intravenously or which are not used at all, to be administered orally.

According to a first aspect of the present invention, there is provided a compound comprising a thiopeptide, or derivative or analogue thereof, the thiopeptide comprising a C-terminal carboxylic acid group, and a functional group for attachment to a drug, characterised in that the compound is adapted to carry or transport a drug.

Preferably, the compound is adapted to, or is capable of carrying or transporting a drug, preferably *in vivo*. The term "drug" as used herein is intended to encompass any pharmaceutically or medicinally active compound or molecule. For example, the drug may have a poor solubility, or may be too polar to cross a membrane when in use. Examples of suitable drugs, which may be used in accordance with the invention may include antivirals, antibiotics, β-blockers, neurotransmitters, hormonal, and anti-cancer drugs. Preferred examples of drugs may include adrenaline, dopamine, GABA, acyclovir, sulfonamides, enalaprilate, burimamide-based H_2 antagonists, propranolol, bestatin, or steroidal drugs.

Advantageously, and preferably, the thiopeptide compound according to the first aspect enables drugs, which are either not used at all in medicine, or which have to be administered intravenously, to be administered to a patient orally. In addition,

advantageously, the thiopeptide compound improves the performance of drugs, which may be normally administered orally, but which may exhibit reduced or low levels of medicinal activity when taken orally, such as drugs with poor solubility. Administering drugs orally, i.e. by mouth, is much simpler and less invasive than by intravenous injection, which is very off-putting for the majority of patients. Therefore, advantageously, use of the compound according to the present invention, will greatly increase the number of drugs that can be used, and administered orally.

The inventors have found that the compound according to the present invention may have a drug molecule attached to the functional group of the thiopeptide, thereby forming a 'compound-drug' conjugate. In addition, the inventors have found that this conjugate-drug has improved transportation properties, for example, across the wall of the gut. The inventors do not wish to be bound by any hypothesis, but believe that the conjugate may be transportable, moved or carried from a first site to a second site by an active transport mechanism. An example of an active transport mechanism is a symporter, which may be a proton-dependant symporter. In particular, the inventors believe that such conjugates may be transported via the PepT1 pathway. Accordingly, the compound is preferably adapted to act as a PepT1 substrate.

PepT1 is most strongly expressed in the jejunum of the small intestine. However, PepT1 has also been isolated from the liver, brain, and from the cortex and medulla of the kidneys. Hence, it will be appreciated that the compound in accordance with the invention may be transported in any of the gut, liver, brain, or in the kidneys etc, and as such, the compound may be transported in any of these tissues. However, in a preferred embodiment, the compound according to the invention may be transported across the lining of the gut, for example, in the small intestine, and particularly, in the jejunum.

A second isoform, PepT2, which shares approximately 50% sequence homology with PepT1, has also been found in the kidneys, where it reabsorbs peptides from the glomerular filtrate. Therefore, it will be appreciated that the compound according to the invention will also have the advantageous properties of being able to exploit the PepT2 pathway, being transported thereby.

Brandsch *et al.* (J.Biol.Chem., Vol.273, 3864-3864, 1998), discloses a thio-Phe-Pro thiopeptide and uses thereof in the investigation of the conformational requirements for substrates for PepT1. Brandsch *et al. supra* do not demonstrate attachment of a drug molecule to the thiopeptide, hydrolysis resistance of the thiopeptide, nor any drug carrying or any drug transportation by the thiopeptide. Accordingly, the compound in accordance with the present invention shows significant surprising advantages over the thiopeptide disclosed in Brandsch *et al. supra* due to the ability of the compound according to the first aspect to be attached to a drug via its functional group, and the compound's ability to transport a drug *in vivo*.

Preferably, the thiopeptide comprises at least two amino acids or derivatives or analogues thereof, or at least three amino acids or derivatives or analogues thereof, or at least four amino acids or derivatives or analogues thereof. Hence, the compound may comprise a dipeptide or a tripeptide or derivatives or analogues thereof. Accordingly, the compound may comprise a thiodipeptide or a thiotripeptide or derivatives or analogues thereof. Preferably, the compound comprises a dipeptide.

The amino acids may be selected from the repertoire of twenty amino acids commonly found in proteins. The compound may comprise an acidic or a basic amino acid. The compound may comprise a hydrophobic or a hydrophilic amino acid. Preferably, the compound comprises a serine, aspartate or glutamate residue as the second or C-terminal residue.

The inventors have found that a serine, aspartate, or glutamate residue represents an advantageous means of attaching a drug to the thiopeptide.

Preferably, the thiopeptide comprises at least one thio group, which thio group is preferably attached at, or towards, an N-terminal thereof. However, the thio group may be attached at, or towards, the C-terminal of the thiopeptide. Preferably, the thio group substitutes a carbonyl group on the thiopeptide. Preferably, the oxygen of a peptide bond between adjacent residues of the peptide is replaced by sulphur, to generate the thiopeptide. The thiopeptide may comprise more than one thio group, for example, two or more thio groups. Hence, where the thiopeptide comprises two amino

acids, the oxygen of a peptide bond between the two residues of the peptide is replaced by sulphur, to generate the thiopeptide. Where the thiopeptide comprises three amino acids, the oxygen of a peptide bond between first and second and/or second and third residues of the peptide may be replaced by sulphur, to generate the thiopeptide, and so on.

Advantageously, the effects of the sulphur atom of the at least one thio group in the thiopeptide are that:-

(i) it allows surprisingly efficient binding of a compound-drug conjugate to a transporter;

(ii) it renders the conjugate substantially resistant to hydrolysis, unlike most peptides; and

(iii) it allows surprisingly rapid *in vivo* transport of the conjugate across the wall of the gut into the bloodstream.

It is most preferred that the compound comprises a thiodipeptide, which comprises two amino acid residues linked together. However, the thiodipeptide is not technically speaking a peptide because it may be considered to lack a peptide bond (CONH) where the two amino acid residues are linked to each other. This is because of the presence of the thio group, which substitutes the carbonyl group (CO) on one of the two residues of the resultant thiopeptide forming a CSNH bond. By the term "thiopeptide" used herein, we mean at least two amino acids joined together, comprising at least one thio- functional group. When the compound comprises a dipeptide, the thiopeptide may be considered to comprise no peptide bonds, (i.e. only a CSNH bond).

Where the thiopeptide comprises a plurality of amino acid residues bonded together, preferably, the number of peptide bonds in the peptide is kept to a minimum. Preferably, the thiopeptide comprises less than four peptide bonds, more preferably, less than three peptide bonds, even more preferably, less than two peptide bonds, and most preferably, no peptide bonds. For example, a CSNH bond is formed where a peptide bond would have been formed by the condensation of two amino acids. For example, when the compound comprises a tripeptide, the thiopeptide may be considered to have one or no peptide bonds, and one or two thio (CSNH) bonds.

Advantageously, and preferably, the compound is substantially resistant to hydrolysis, for example, by peptidases, because the thiopeptide compound has either few peptide bonds or no peptide bonds at all, which would otherwise be digested by peptidases. It is preferred that the compound comprises a thiodipeptide, or derivative or analogue thereof, characterised in that a thiopeptide bond substitutes a peptide (CONH) bond, wherein the C-terminal residue of the peptide comprises serine.

It is preferred that the compound comprises a thiodipeptide, or derivative or analogue thereof, characterised in that a thio (CSNH) bond substitutes a peptide (CONH) bond, wherein the C-terminal residue of the peptide comprises an acidic amino acid, for example, aspartate or glutamate.

The compound may have formula I:-

$$H_2N - CR^1R^2 - CS - NR^3 - CR^4R^5 - COX$$

wherein R^1, R^2, R^3, R^4, and R^5 may be independently selected from a group consisting of a hydrogen; a linear or branched alkyl group; a dialkyl group; a N-alkyl group; and a side chain group of an amino acid residue; and wherein X may be independently selected from a hydroxyl group; an amino acid residue; an amide; an amide link to a third residue; a peptide; and a thiopeptide.

It is preferred that R^2 may be hydrogen. It is preferred that R^3 may be hydrogen. It is preferred that R^5 may be hydrogen. It is preferred that X may be a hydroxyl group.

Hence, in a preferred embodiment, the compound may have formula II:-

$$H_2N - CHR^1 - CS - NH - CHR^4 - COOH$$

wherein R^1, and R^4 may be independently selected from a group consisting of a hydrogen; a linear or branched alkyl group; a dialkyl group; a N-alkyl group; and a side chain group of an amino acid residue.

Preferably, R^4 is adapted to be attached to a drug molecule. In a preferred embodiment, R^4 comprises an alcohol or a carboxylic acid group. Preferably, R^4 comprises an alkyl chain attached to an alcohol or a carboxylic acid group. It is preferred that R^4 is an amino acid side chain comprising an alcohol or a carboxylic acid group. Hence, in a preferred embodiment, R^4 is an amino acid side chain, which incorporates either an alcohol or a carboxylic group.

Advantageously, and preferably, when a drug molecule is attached to the R^4 group of the thiopeptide, the thiopeptide comprises a C-terminal COOH group, which is preferred for substrate recognition by PepT1 protein, and transportation thereby. The Example and Figure 2 shows a structure of a substrate for PepT1, which preferably comprises a thiodipeptide with a C-terminal carboxylic acid group.

The alkyl group or alkyl chain may comprise a C_1-C_{20} chain, and preferably, a C_1-C_{15} chain. It is envisaged that the alkyl group or the alkyl chain comprises a C_1-C_{10} chain, and more preferably, a C_1-C_6 chain, and most preferably a C_1-C_3 chain. The alkyl group or alkyl chain may be a methyl, propyl, butyl, or a pentyl chain.

In a preferred embodiment, $R^1$and R^4 may be a side chain group of any amino acid residue. It is envisaged that $R^1$and R^4 may be the same as each other, or different from each other.

The amino acid side chain group may be independently selected from an amino acid side chain group of an acidic, basic, hydrophobic or a hydrophilic amino acid residue. For example, the amino acid side chain group may be independently selected from a group consisting of (i) H (glycine); (ii) Me (alanine); (iii) CH_2Ph (phenylalanine); (iv) $CHMe_2$ (valine); (v) CH_2OH (serine); (vi) CH_2SH (cysteine); (vii) CH_2CO_2H (aspartate); (viii) CH_2CONH_2 (asparagine); and (ix) $(CH_2)_4NH_2$ (lysine).

In a preferred embodiment, R^1 is selected from any of the side chain groups shown in column D in Figure 1.

The functional group to which a drug is attached may be protected by a protection group. Suitable protection groups include t-butyl, benzyl, methoxybenzyl, allyl or fluorenyl attached to nitrogen, oxygen or sulphur via ester, thioether, or carbamate linkages. The functional group may be deprotected before attachment of a drug to the compound and this may be achieved by the addition of Na/NH_3 (liquid), thereby freeing up the functional group ready for attachment of the drug.

Derivatives or analogues of the thiopeptide compound according to the invention may include derivatives or analogues that increase or decrease the peptide's half-life *in vivo*. Examples of derivatives or analogues capable of increasing the half-life of the peptide according to the invention include peptoid derivatives, D-amino acid derivatives of the peptides, and peptide-peptoid hybrids.

The thiopeptide according to the invention may be subject to degradation by a number of means (such as protease activity in biological systems). Such degradation may limit the bioavailability of the peptide, and hence the ability of the peptide to achieve its biological function. There are wide ranges of well-established techniques by which peptide derivatives or analogues that have enhanced stability in biological contexts can be designed and produced. Such peptide derivatives may have improved bioavailability as a result of increased resistance to protease-mediated degradation. Preferably, a peptide derivative or analogue suitable for use according to the invention is more protease-resistant than the peptide from which it is derived. Protease-resistance of a peptide derivative and the peptide from which it is derived may be evaluated by means of well-known protein degradation assays. The relative values of protease resistance for the peptide and the peptide derivative or analogue may then be compared.

Peptoid derivatives of the thiopeptide of the invention may be readily designed from knowledge of the structure of the peptide. Peptoid compounds have two properties that make them suitable for use as peptide derivatives/analogues according to the invention:-

(i) In peptoid residues, no hydrogen bond involving the NH would be possible.

(ii) The peptoids are resistance to enzymatic degradation.

Commercially available software may be used to develop peptoid derivatives according to well-established protocols.

Retropeptoids, (in which all amino acids are replaced by peptoid residues in reversed order) are also able to mimic peptides. A retropeptoid is expected to bind in the opposite direction in the ligand-binding groove, as compared to a peptide or peptoid-peptide hybrid containing one peptoid residue. As a result, the side chains of the peptoid residues are able to point in the same direction as the side chains in the original peptide.

A further embodiment of a modified form of peptide according to the invention comprises D-amino acid forms of the peptide. The preparation of peptides using D-amino acids rather than L-amino acids greatly decreases any unwanted breakdown of such an agent by normal metabolic processes, decreasing the amounts of agent which need to be administered, along with the frequency of its administration.

According to a second aspect of the present invention, there is provided a drug carrier comprising a thiopeptide, or derivative or analogue thereof.

Preferably, the thiopeptide, or derivative or analogue thereof comprises the thiopeptide, or derivative or analogue thereof according to the first aspect. Preferably, the thiopeptide, or derivative or analogue thereof, comprises a C-terminal carboxylic acid group, and preferably, a functional group for attachment to a drug. Preferably, the compound is adapted to carry or transport a drug. It is preferred that the drug carrier comprises a thiodipeptide or a thiotripeptide. Preferably, a C-terminal amino acid residue of the thiopeptide is adapted to be attached to a drug molecule. Preferably, the functional group is present at, or towards, the C-terminal of the thiopeptide.

The amino acids may be selected from the repertoire of twenty amino acids commonly found in proteins, or other non-DNA encoded amino acids. The compound may comprise an acidic or a basic amino acid. The compound may comprise a hydrophobic or a hydrophilic amino acid. Preferably, the compound comprises a

serine, glutamate or an aspartate residue, preferably as the second residue, for example, when the thiopeptide is a dipeptide. Preferably, the thiopeptide comprises at least one thio group, which may be present on the N-terminal residue, preferably substituting a carbonyl group of the peptide.

Preferably, the functional group comprises an alcohol or a carboxylic group which is adapted to be attached to a drug molecule.

The drug carrier may have a formula I, and preferably formula II according to the first aspect.

According to a third aspect of the invention, there is provided a drug conjugate comprising a drug, which drug is linked to a compound according to the first aspect or a drug carrier according to the second aspect.

Hence, the compound according to the first aspect or the drug carrier according to the second aspect may be adapted to be attached to a drug molecule, thereby forming a 'compound-drug' or a 'drug carrier-drug' conjugate, hereinafter referred to as a 'drug conjugate'. Preferably, the attachment of the drug to the compound or drug carrier is by covalent bonding. It will be appreciated by the skilled technician that covalent bonding between the drug molecule and the compound according to the first aspect or the drug carrier according to the second aspect may be achieved by reacting a functional or reactive group on the compound or drug carrier, and a functional or reactive group on the drug. Preferably, the compound or drug carrier comprises at least one functional group, which functional group is adapted to react with the drug molecule. The functional group on the compound or drug carrier may be present on the N-terminal or C-terminal residue of the thiopeptide. The skilled technician will appreciate the types of functional or reactive groups, which would react with the drug molecule. For example, the at least one functional group may be an oxygen group, a carbonyl group, a hydroxyl group or a carboxylic acid group, which may be present on either the first or second amino acid residue.

It should be appreciated that the present invention does not extend to the selection of the drug itself. The inventors did not investigate the biological activity of

any drug molecule being attached to the compound according to the first aspect or the carrier according to the second aspect. The inventors tested a number of different drug analogue molecules or 'test' molecules to investigate the efficacy of binding said analogue molecules to the compound according to the first aspect or the carrier according to the second aspect, to thereby form the conjugate. They then tested the transportation of the conjugate. These drug analogue molecules did not have any biological activity, as they were merely test molecules. However, these drug analogue molecules or 'test' molecules did resemble biologically active drug molecules. Therefore, it will be appreciated that it would be preferred to attach a biologically active drug molecule to the compound according to the first aspect or the carrier according to the second aspect.

Examples of different drug analogue 'test' molecules, which were attached to compound according to the first aspect or the carrier according to the second aspect are shown in columns B and C of Figure 1 as Y.

Preferably, the drug comprises at least one functional group with which the functional group of the compound or drug carrier may react. As with above, the skilled technician will appreciate the types of functional or reactive groups, which would react with the compound or drug carrier. For example, the functional group on the drug molecule may comprise a carboxylic acid group or a hydroxyl group. Preferably, attachment of the drug to the compound of the first aspect or the drug carrier of the second aspect is by means of an ester linkage. Preferably, attachment of the drug occurs at residue 1 or 2 of the compound or drug carrier.

It is envisaged that attachment of the drug to the compound according to the first aspect or the drug carrier according to the second aspect may be by means of an ester linkage, or an ether linkage, or an amide linkage.

Changes to any of R^1, R^2, R^3, R^4, and/or R^5, may make it possible to modify the compound or drug carrier in accordance with the invention so that any adverse features on the drug may be minimised by the nature of the first residue of the thiopeptide. Preferably, R^1 on the first amino acid residue (N-terminal) is suitable for such modification. For example, it may be beneficial to modify the net charge of the

compound according to the first aspect, or the drug carrier according to the second aspect, such that the conjugate is pharmaceutically acceptable for use in medicine. For example, if the drug being attached to the compound or drug carrier is acidic, then it may be advantageous to neutralise the net charge of the compound-drug conjugate by using a basic compound or drug carrier. Hence, the net charge of the compound or drug carrier may be modulated by selection and/or modification of any of R^1, R^2, R^3, R^4, and/or R^5 groups. Preferably, the net charge may be modulated by selection and/or modification of the R^1 group.

It is also envisaged that it could be possible to selectively detach the drug molecule from the conjugate, by designing the compound or drug carrier so that the linkage between the compound/drug carrier and drug can be broken when the conjugate reaches its target environment or position *in vivo*. For example, it is possible to design the thiopeptide drug carrier with appropriate amino acid residues such that the linkage with the drug can be broken when the conjugate is present in an acidic environment, for example, an area of wound tissue, which may have a low pH. Alternatively, some enzymes may only be expressed or be fully functional in certain tissues, and the thiopeptide-drug bond may be digested by such enzymes, when the conjugate reaches that particular tissue(s).

Accordingly, the compound according to the first aspect or the drug carrier according to the second aspect may be capable of being released or detached from the drug molecule.

Preferably, the compound is in the form of an L-isomer. Preferably, the or each amino acid is an L-isomer. Advantageously, use of an L-isomer improves the binding between the thiopeptide and the drug.

According to a fourth aspect of the invention, there is provided a conjugate according to the third aspect, for use as a medicament.

Preferably, there is provided use of the conjugate according to a third aspect for the preparation of an orally administrable medicament.

Preferably, the medicament or conjugate is orally administered to an individual. The medicament or conjugate may be adapted to be transported into the bloodstream via a PepT1/T2 pathway.

It will be appreciated that the conjugate according to the third aspect of the present invention may be used in a monotherapy (i.e. use of the compound or derivatives thereof according to the invention alone). Alternatively, the conjugate according to the invention may be used as an adjunct, or in combination with, known therapies.

The conjugate according to the invention may be combined in compositions having a number of different forms depending, in particular on the manner in which the composition is to be used. Thus, for example, the composition may be in the form of a powder, tablet, capsule, liquid, gel, hydrogel, aerosol, spray, micelle, liposome or any other suitable form that may be administered to a person or animal. It will be appreciated that the vehicle of the composition of the invention should be one which is well tolerated by the subject to whom it is given, and preferably enables oral delivery of the compound.

Compositions comprising the conjugate according to the invention may be used in a number of ways. For instance, systemic administration is preferred, in which case the conjugate may be contained within a composition that is preferably ingested orally in the form of a tablet, capsule or liquid. Alternatively, it is possible that the composition may be administered by inhalation (e.g. intranasally). In some circumstances, the composition may be administered by injection into the blood stream. Injections may be intravenous (bolus or infusion) or subcutaneous (bolus or infusion).

The conjugate may also be incorporated within a slow or delayed release device. Such devices may, for example, be ingested and retained in the gut, and the conjugate may be released over weeks or even months. Such devices may be particularly advantageous when long-term treatment with the conjugate according to the invention is required and which would normally require frequent administration (e.g. at least daily injection).

Due to the 1:1 stoichiometry of the drug:conjugate, it will be appreciated that the amount of conjugate, and therefore drug, required in the conjugate according to the present invention, is determined by its biological activity and bioavailability, which in turn depends on the mode of administration, the physicochemical properties of the drug employed, and whether the drug is being used as a monotherapy or in a combined therapy. The frequency of administration will also be influenced by the above-mentioned factors and particularly the half-life of the conjugate within the subject being treated.

Optimal dosages to be administered may be determined by those skilled in the art, and will vary with the particular conjugate/drug in use, the strength of the preparation, the mode of administration, and the advancement of the disease condition. Additional factors depending on the particular subject being treated will result in a need to adjust dosages, including subject age, weight, gender, diet, and time of administration.

Known procedures, such as those conventionally employed by the pharmaceutical industry (e.g. *in vivo* experimentation, clinical trials, etc.), may be used to establish specific formulations of the compound according to the invention, and precise therapeutic regimes (such as daily doses and the frequency of administration).

Generally, a daily dose of between 0.01 μg/kg of body weight and 1.0 g/kg of body weight of the conjugate according to the invention may be used for the prevention and/or treatment of the specific medical condition. More preferably, the daily dose is between 0.01 mg/kg of body weight and 100 mg/kg of body weight. Daily doses may be given as a single administration (e.g. a single daily tablet). Alternatively, the conjugate may require administration twice or more times during a day. As an example, the conjugate according to the invention may be administered as two (or more depending upon the severity of the condition) daily doses of between 25 mgs and 5000 mgs. A patient receiving treatment may take a first dose upon waking and then a second dose in the evening (if on a two dose regime) or at 3 or 4 hourly

intervals thereafter. Alternatively, a slow release device may be used to provide optimal doses to a patient without the need to administer repeated doses.

This invention provides a pharmaceutical composition comprising a therapeutically effective amount of a drug and the compound or drug carrier according to the present invention. In one embodiment, the amount of the conjugate is an amount from about 0.01 mg to about 800 mg. In another embodiment, the amount of the conjugate is an amount from about 0.01 mg to about 500 mg. In another embodiment, the amount of the conjugate is an amount from about 0.01 mg to about 250 mg. In another embodiment, the amount of the conjugate is an amount from about 0.1 mg to about 60 mg. In another embodiment, the amount of the conjugate is an amount from about 0.1 mg to about 20 mg.

This invention provides a process for making a pharmaceutical composition comprising combining a therapeutically effective amount of a drug, the compound or drug carrier according to the present invention, and a pharmaceutically acceptable vehicle. A "therapeutically effective amount" is any amount of a drug which, when administered to a subject provides prevention and/or treatment of a specific medical condition. A "subject" is a vertebrate, mammal, domestic animal or human being.

A "pharmaceutically acceptable vehicle" as referred to herein is any physiological vehicle known to those of ordinary skill in the art useful in formulating pharmaceutical compositions. Preferably, the pharmaceutically acceptable vehicle, is adapted for oral administration. In a preferred embodiment, the pharmaceutical vehicle is a liquid and the pharmaceutical composition is in the form of a solution. In another embodiment, the pharmaceutically acceptable vehicle is a solid and the composition is in the form of a powder or tablet. In a further embodiment, the pharmaceutical vehicle is a gel and the composition is in the form of a cream or the like.

A solid vehicle can include one or more substances, which may also act as flavouring agents, lubricants, solubilisers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material. In powders, the vehicle is a finely divided solid that is in

admixture with the finely divided active drug. In tablets, the drug is mixed with a vehicle having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain up to 99% of the active drug. Suitable solid vehicles include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, polyvinylpyrrolidine, low melting waxes and ion exchange resins.

Liquid vehicles are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active drug can be dissolved or suspended in a pharmaceutically acceptable liquid vehicle such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid vehicle can contain other suitable pharmaceutical additives such as solubilisers, emulsifiers, buffers, preservatives, sweeteners, flavouring agents, suspending agents, thickening agents, colours, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid vehicles for oral and parenteral administration include water (partially containing additives as above, e.g. cellulose derivatives, preferably sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g. glycols) and their derivatives, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the vehicle can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid vehicles are useful in sterile liquid form compositions for parenteral administration. The liquid vehicle for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellent.

In some cases, where it is desired to inject the conjugate, liquid pharmaceutical compositions which are sterile solutions or suspensions can be utilized by for example, intramuscular, intrathecal, epidural, intraperitoneal, intravenous and particularly subcutaneous, intracerebral or intracerebroventricular injection. The drug may be prepared as a sterile solid composition that may be dissolved or suspended at the time of administration using sterile water, saline, or other appropriate sterile injectable medium. Vehicles are intended to include necessary and inert binders, suspending agents, lubricants, flavourants, sweeteners, preservatives, dyes, and coatings.

The conjugant according to the invention can be administered orally in the form of a sterile solution or suspension containing other solutes or suspending agents (for example, enough saline or glucose to make the solution isotonic), bile salts, acacia, gelatin, sorbitan monoleate, polysorbate 80 (oleate esters of sorbitol and its anhydrides copolymerized with ethylene oxide) and the like. Preferably, the conjugate according to the invention is administered orally either in liquid or solid composition form. Compositions suitable for oral administration include solid forms, such as pills, capsules, granules, tablets, and powders, and liquid forms, such as solutions, syrups, elixirs, and suspensions. Forms useful for parenteral administration include sterile solutions, emulsions, and suspensions.

According to a fifth aspect, there is provided an assay adapted to detect transportation of a conjugate according to the third aspect from a first side of a membrane to a second side of a membrane, the assay comprising detection means adapted to detect the presence of the conjugate on first and second sides of the membrane.

The membrane may be present in the stomach, liver, brain, or the kidneys. Preferably, the membrane is present in the intestine, more preferably, the small intestine and most preferably, the jejunum of the small intestine.

The detection means may be adapted to detect UV absorption of the thiopeptide, preferably, high UV absorption thereof. The UV absorption may be detected by, for example, using HPLC. In this way, the detection means may be used to detect a thiopeptide conjugate in cells or vesicles.

The compound according to the first aspect of the invention or the drug carrier according to the second aspect of the invention may be synthesised using common known chemical synthesis techniques. An example method for synthesising the compound or drug carrier according to the invention is disclosed in Figures 3 and 4, and is discussed in the Example.

It will be appreciated by the skilled technician that there are many ways that the compound and drug carrier according to the invention could be made. However,

the sulphur atom of the thio- functional group causes potential problems with the molecule, and the method disclosed herein provides an effective solution. It will be appreciated that small changes to any of the steps of the synthesis disclosed herein may be made while still benefiting from the invention.

According to a sixth aspect, there is provided a method of treating an individual, the method comprising administering to an individual in need of such treatment, a drug conjugate according to the third aspect.

Preferably, the drug conjugate comprises a drug molecule attached to a compound according to the first aspect or a drug carrier according to the second aspect.

All of the features described herein (including any accompanying claims, abstract and drawings), and/or all of the steps of any method or process so disclosed, may be combined with any of the above aspects in any combination, except combinations where at least some of such features and/or steps are mutually exclusive.

For a better understanding of the invention, and to show how embodiments of the same may be carried into effect, reference will now be made, by way of example, to the accompanying drawings, in which:-

Figure 1 represents a Table showing variations in thiopeptide conjugates that might be tested in accordance with the third aspect of the invention;

Figure 2 shows a schematic diagram for the preparation of a compound according to the first aspect of the invention or a drug carrier according to the second aspect of the invention;

Figure 3 shows a schematic diagram for the synthesis of a protected thiopeptide serine drug carrier in accordance with the invention;

Figure 4 shows a schematic diagram for the synthesis of a drug conjugate in accordance with the third aspect of the present invention; and

Figure 5 shows a bar graph showing binding data for a drug carrier according to the second aspect of the invention;

Example 1

The aim of the present invention was to make a drug carrier compound, which enables the preparation of drug-drug carrier conjugates, which would allow the oral administration of drugs that are not currently administrable by mouth. In addition, such drug-drug carrier conjugates could also be used to improve the efficacy of drugs which are currently administered orally, but which show decreased levels of biological activity when administered by mouth. In order to achieve this aim, the following steps were carried out:-

a) A range of thiopeptide drug carrier analogues were prepared;
b) Methods for synthesising the drug carriers were developed, both to facilitate the rapid synthesis of a wide range of analogues, and also to enable the preparation of the drug carrier cheaply and on a large scale;
c) Bio-assays were developed to test the efficacy of the drug carrier analogues;
d) A transport bioassay was developed that works quickly, and with small amounts of the drug substrate;
e) The *in vivo* transportation of a non-orally available medicine by PepT1 across the lining of the gut was demonstrated.

1) Preparation of a range of thiopeptide carrier molecules

The inventors based their work on a 3D substrate model of a substrate, shown as structure A, for the PepT1 protein, which is shown in Figure 2. While attempting to refine the proposed PepT1 substrate model shown in Figure 2, a thiopeptide analogue drug carrier as shown as structure B in Figure 2 was prepared. The inventors were surprised to discover that Structure B exhibits three main advantageous features namely (i) it binds efficiently to PepT1 (Ki 0.3mM, typical natural substrates, *in vitro* assay); (ii) it is rapidly transported *in vivo*; and (iii) it is resistant to hydrolysis, unlike most peptides. Hence, the thiopeptide shown as structure B in Figure 2 was believed to be a potential carrier for drugs that do not naturally diffuse across the villi of the small intestine, or that have poor solubility, provided that a method of attaching a drug to compound B at position X, could be devised. Accordingly, serine, aspartate or glutamate analogues of compound C as shown in Figure 2 were made, in which X is hydroxy or carboxylic acid group, connected to the drug via an ester linkage, thereby forming a drug conjugate.

2) Preparation of a protected thiopeptide serine drug carrier

A method was then devised for preparing a set of thiopeptide carriers that are suitably protected for the attachment of drugs that possess a carboxylic acid reactive group. The method of synthesising a protected thiopeptide serine drug carrier compound is summarized in Figure 3. The particular strategy and choice of protecting groups were designed to enable the preparation of large amounts of drug carrier molecules, to which a drug molecule could then be attached. Suitable protection groups include t-butyl, benzyl, methoxybenzyl, allyl or fluorenyl attached to nitrogen, oxygen or sulphur via ester, thioether, or carbamate linkages.

The final step before attachment of a drug to the carrier molecule, is the deprotection of the functional group on the carrier to which the drug molecule is to be attached. This is achieved by the addition of Na/NH_3 (liquid), thereby freeing up the functional group ready for attachment of the drug.

Following attachment of the drug to the drug carrier/compound in accordance with the present invention, the conjugate was then globally deprotected. This method has enabled the preparation of a large range of drug-carrier conjugates very quickly.

3) Preparation of a drug conjugate

Referring to Figure 4, there is shown the successful attachment of a range of carboxylic acids, which acted as 'test' drug molecules to the drug carrier thiopeptide produced in (2) above. Following attachment of the carboxylic acid 'test' molecule to the drug carrier, the conjugate was then deprotected so that the efficacy of transportation could be investigated. Conjugates A, B, D and E shown in Figure 4 have been demonstrated to all bind well to PepT1 (see Results section below). In addition, actual transportation of analogue E by the PepT1 pathway has also been shown (see Results section below).

4) Results

1) *In vivo* transport experiments using the methodology described in Lister *et al.* (*J. Physiol.*, 1995, 484.1, 173–182), i.e. actual direct measurements of transport of thiopeptides across the gut wall, using rat jejenum, indicated that Phe–ΨS–Ala (i.e. the

thiodipeptide of phenylalanine and alanine), is transported more efficiently than D-Phe-L-Ala (a standard, non-hydrolyzable dipeptide), as detected by HPLC analysis:-

a) 1mM D-Phe-L-Gln is transported at 0.244 (+/- 0.036) micromol/min/g of whole dry intestine.

b) 1mM of the thiopeptide analogue of L-Phe-L-Ala is transported at 3.09 (+/- 0.047) micromol/min/g of whole dry intestine.

This indicates not only that the thiopeptide according to the invention is recognized by PepT1, but also :-

(a) that the PepT1 transport mechanism still operates; and

(b) that the thiopeptide is resistant to the peptidases that are present in and around the villi of the small intestine.

The data shows that the thiopeptide is therefore transported at about 13 times the rate of the dipeptide D-Phe-L-Gln.

2) Quantitative binding data has been produced for the five thiodipeptide carriers with attached drug surrogates using the methodology described in Meredith *et al.* (*J. Physiol.*, 1998, 512, 629–634) and is shown in Table 2 below. These five compounds are E (209), B (210), A (211), D (212) and C (213), as shown in Figure 4.

Table 2 – Binding data for thiopeptide carriers

Compound number	**K_i**
209	0.30 +/- 0.06mM
210	0.36 +/- 0.11mM
211	0.10 +/- 0.04mM
212	0.027 +/- 0.006mM
213	0.60 +/- 0.13mM

The data are also presented in the bar chart illustrated as Figure 5.

3) Compound 209 (E) was then tested for transport using a trans-stimulation assay using the methodology described in Temple (*J. Biol. Chem.*, 1998, 273, 20–22), from which the efflux of radio-labelled D-Phe-L-Gln indicated that it was indeed

transported. There are no reports in the literature of thiodipeptides attached to drugs or drug analogues, and therefore no examples of such conjugates being actively transported.

The inventors of the present invention have shown that Compound 209 (E):-

a) Inhibits uptake of D-Phe-L-Gln by 88% (+/- 1.4) at 1 nM, giving a K_i of 0.3 mM (+/- 0.06); and

b) At 2mM, Compound 209 (E) gave 54% efflux compared to 72% for 10 mM Gly-Gln, indicating that it is transported more efficiently than Gly-Gln.

The transport data was measured as average cpm (counts per minute) from 5 experiments:-

Control 4852 (+/- 89) cpm remaining in oocytes

10 mM Gly-Gln 1347 (+/- 126)

2 mM conjugate 2237 (+/- 594)

In addition to these aforementioned thiopeptide analogues, additional analogues were prepared as shown in Figure 1. It should be noted that two of the modifications (structure B in column A, and all of column D of Figure 1) relate to the drug carrier compound itself, and therefore necessitate changes early in the synthesis. These latter two modifications are important from a drug delivery standpoint, because changes to the side-chain of the first amino acid residue in the thiopeptide allows the 'fine-tuning' of the drug delivery system so that any adverse features on the drug are off-set by the nature of amino acid residue 1. For example, the preferred overall neutrality of the drug-drug carrier conjugate at pH5.5. Changing the second amino acid residue to aspartate enables the attachment of drugs possessing a hydroxyl function, which leads to a massive increase in the potential number of drugs that might be suitable for attachment to the carrier.

In conclusion, the experiments and data described herein illustrate the efficacy of the thiopeptide compound in accordance with the invention as a drug carrier molecule, and how drug-carrier conjugates may be transported across membranes *in vivo* using the PepT1 protein.

CLAIMS

1. A compound comprising a thiopeptide, or derivative or analogue thereof, the thiopeptide comprising a C-terminal carboxylic acid group, and a functional group for attachment to a drug, characterised in that the compound is adapted to carry or transport a drug.

2. A compound according to claim 1, wherein the compound is adapted to, or is capable of carrying or transporting a drug, preferably *in vivo*.

3. A compound according to either claim 1 or claim 2, wherein the compound is adapted to be transported by a PepT1 protein or a PepT2 protein.

4. A compound according to any preceding claim, wherein the thiopeptide comprises at least two amino acids or derivatives or analogues thereof.

5. A compound according to any preceding claim, wherein the compound comprises a thiodipeptide or a thiotripeptide or derivatives or analogues thereof.

6. A compound according to any preceding claim, wherein the compound comprises a serine, aspartate or glutamate residue as a C-terminal residue.

7. A compound according to any preceding claim, wherein the thiopeptide comprises at least one thio group, which thio group is attached at, or towards, an N-terminal thereof.

8. A compound according to any preceding claim, the compound has formula I:-

$$H_2N - CR^1R^2 - CS - NR^3 - CR^4R^5 - COX \qquad (I)$$

wherein R^1, R^2, R^3, R^4, and R^5 are independently selected from a group consisting of a hydrogen; a linear or branched alkyl group; a dialkyl group; a N-alkyl group; and a side chain group of an amino acid residue; and wherein X is independently selected

from a hydroxyl group; an amino acid residue; an amide; an amide link to a third residue; a peptide; and a thiopeptide.

9. A compound according to any of claims 1 to 7, wherein the compound has formula II:-

$$H_2N - CHR^1 - CS - NH - CHR^4 - COOH \qquad (II)$$

wherein R^1, and R^4 are independently selected from a group consisting of a hydrogen; a linear or branched alkyl group; a dialkyl group; a N-alkyl group; and a side chain group of an amino acid residue.

10. A compound according to either claim 8 or claim 9, wherein R^4 is adapted to be attached to a drug molecule.

11. A compound according to any of claims 8 to 10, wherein R^4 comprises an alcohol or a carboxylic acid group.

12. A compound according to any of claims 8 to 11, wherein R^4 comprises an alkyl chain attached to an alcohol or a carboxylic acid group.

13. A compound according to any of claims 12, wherein the alkyl group or alkyl chain comprises a C_1-C_{20} chain.

14. A compound according to any of claims 8 to 13, wherein R^4 is an amino acid side chain group comprising an alcohol or a carboxylic acid group.

15. A compound according to any of claims 8 to 14, wherein $R^1$and R^4 is a side chain group of any amino acid residue.

16. A compound according to either claim 14 or claim 15, wherein the amino acid side chain group is independently selected from a group consisting of (i) H (glycine); (ii) Me (alanine); (iii) CH_2Ph (phenylalanine); (iv) $CHMe_2$ (valine); (v) CH_2OH

(serine); (vi) CH_2SH (cysteine); (vii) CH_2CO_2H (aspartate); (viii) CH_2CONH_2 (asparagine); and (ix) $(CH_2)_4NH_2$ (lysine).

17. A compound according to any preceding claim, wherein a functional group to which a drug is attached is protected by a protection group.

18. A drug carrier comprising a thiopeptide, or derivative or analogue thereof.

19. A drug carrier according to claim 18, wherein the thiopeptide, or derivative or analogue thereof comprises the thiopeptide, or derivative or analogue thereof according to any of claims 1 to 17.

20. A drug conjugate comprising a drug, which drug is linked to a compound according to any of claims 1 to 17, or a drug carrier according to either claim 18 or claim 19.

21. A drug conjugate according to claim 20, wherein attachment of the drug to the compound or drug carrier is by means of an ester linkage, ether linkage or an amide linkage.

22. A drug conjugate according to either claim 20 or claim 21, wherein attachment of the drug occurs at residue 1 or 2 of the compound or drug carrier.

23. A drug conjugate according to any of claims 20 to 22, wherein the compound or the drug carrier is capable of being released or detached from the drug molecule.

24. A drug conjugate according to any of claims 20 to 23, for use as a medicament.

25. Use of the conjugate according to any of claims 20 to 23 for the preparation of an orally administrable medicament.

26. An assay adapted to detect transportation of a conjugate according to any of claims 20 to 23 from a first side of a membrane to a second side of a membrane, the

assay comprising detection means adapted to detect the presence of the conjugate on first and second sides of the membrane.

27. An assay according to claim 26, wherein the detection means is adapted to detect UV absorption of the thiopeptide.

28. A method of treating an individual, the method comprising administering to an individual in need of such treatment a conjugate according to any of claims 20 to 23.

29. A method of treating an individual according to claim 28, wherein the drug conjugate comprises a drug molecule attached to a compound according to any of claims 1 to 17 or a drug carrier according to either claim 18 or 19.

30. A compound or drug carrier substantially as herein described with reference to, or as illustrated by, the figures.

31. A method substantially as herein described with reference to, or as illustrated by, the figures.

Figure 1

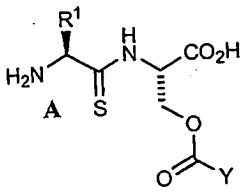
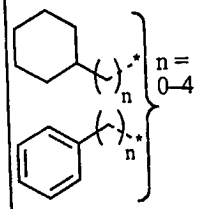
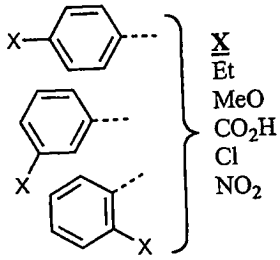
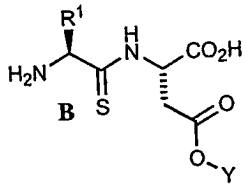
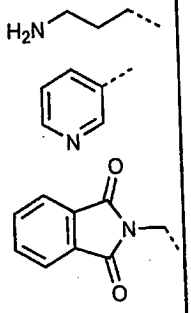
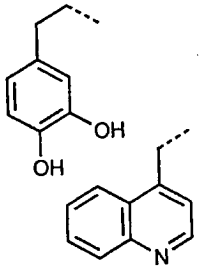
Column A Thiodipeptide core	Column B Y gps (*cf.* drugs)	Column C Y gps (*cf.* drugs)	Column D Side-chain R^1 gps
 A	 n = 0–4	 X Et MeO CO_2H Cl NO_2	H Me CH_2Ph $CHMe_2$ CH_2OH CH_2SH CH_2CO_2H CH_2CONH_2 $(CH_2)_4NH_2$
 B			

Figure 2.

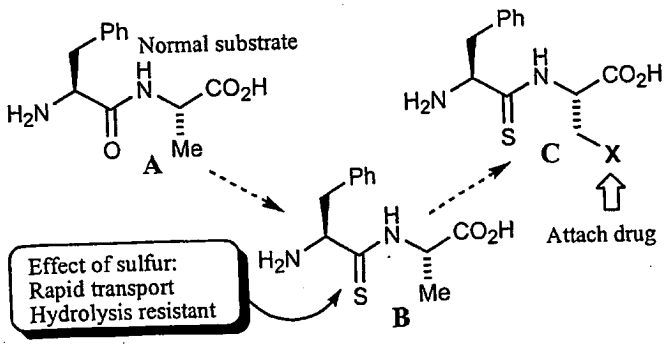


Figure 3

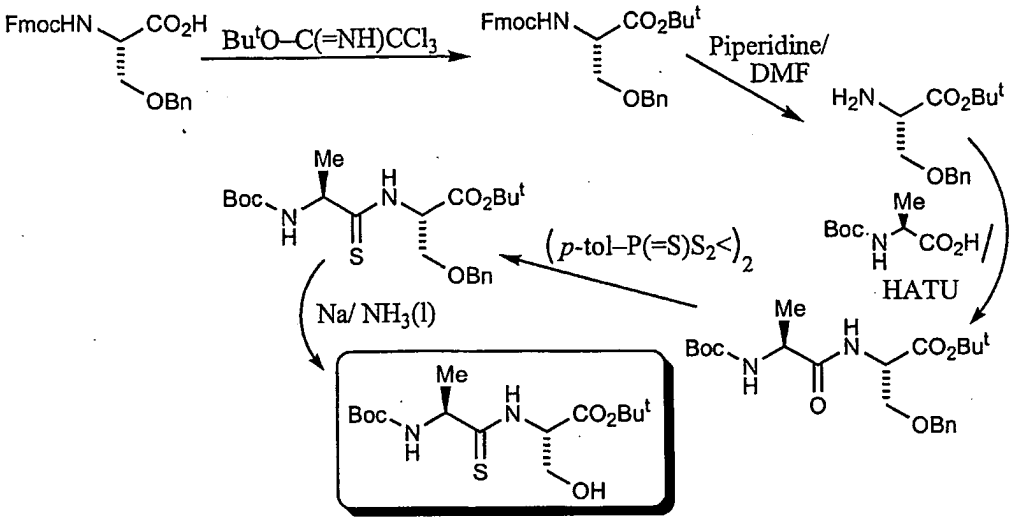


Figure 4

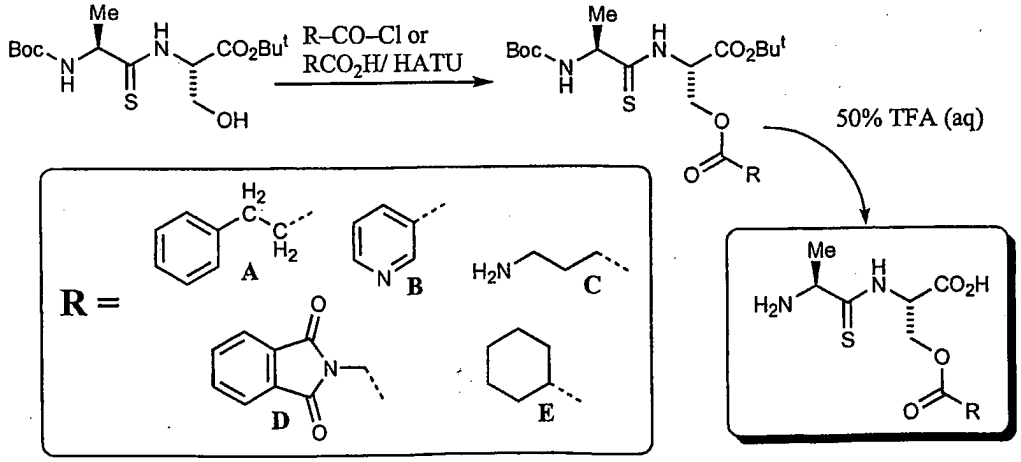
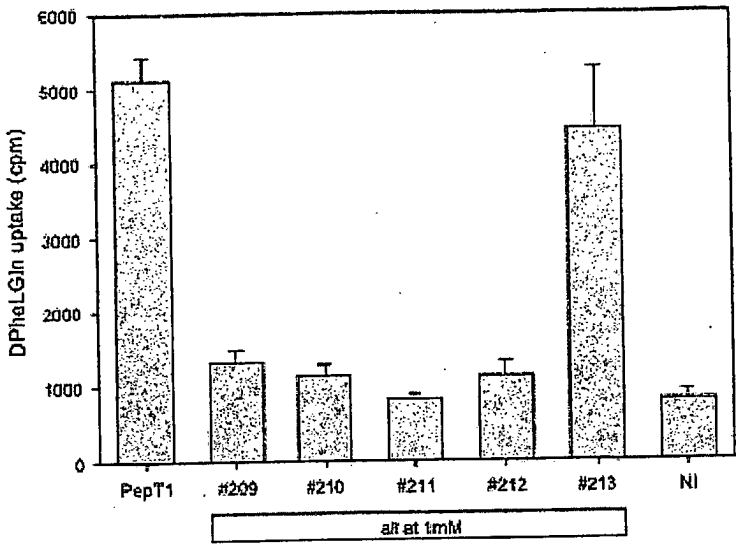


Figure 5


6000
5000
4000
3000
2000
1000
0
DPheLGln uptake (cpm)
PepT1
#209
#210
#211
#212
#213
NI
all at 1mM